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TITLE: Training Program in Breast Cancer Research

PRINCIPAL INVESTIGATOR: John Shively, Ph.D.

CONTRACTING ORGANIZATION: City of Hope National Medical Center
Duarte, California 91010-3000

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13. ABSTRACT (Maximum 200 Words)

The objective of the City of hope breast cancer training program is to develop a new generation of basic and clinical scientists trained to do research on breast cancer and its prevention. The program will draw predoctoral trainees from the CoH graduate school and post doctoral trainees from the basic sciences and clinical oncology disciplines at CoH. Those who show a genuine interest in breast cancer research, as determined by a written application, are admitted into the program in a competition held each year for available slots. Trainees are required to work in labs that study fundamentals of cancer biology and are required to develop a research program that focuses on a specific problem in breast cancer. In addition to performing their breast cancer research, the fellows are required to attend and participate in courses on 1) the biology and pathology of breast cancer, 2) breast cancer prevention and treatment, 3) the ethical conduct of basic and clinical research, 4) biostatistics, 5) genetic predisposition of breast cancer and counseling, and 6) the quality of life and pain management in breast cancer. Trainees attend a biweekly seminar/journal club course in which they present their research and one recent paper to each and their mentor. They receive a critical review of both presentations and benefit from the others presentations on breast cancer.

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PROGRESS REPORT

Title: Training program in breast cancer
Number: DAMD17-00-1-0203
Principle Investigator: John E. Shively

Introduction:
    N/A

Body:

Task 1. Recruit trainees into the breast cancer training program. Each year we determine the number of available slots, advertise the competition across the entire campus, and solicit new applications. The competition is open to both predoctoral and postdoctoral fellows. This year we had two new openings. A total of nine applicants applied. Each wrote a 5 page research proposal that was read and scored by the Internal Advisory Committee. Two applicants were selected and began their fellowships on 9/1/03. We have a total of 7 fellows who finished the program when it expired on 9/1/04.

Task 2. Assign trainees to laboratories and identify breast cancer related projects. This was accomplished as described above.

Task 3. Initiate coursework, journal clubs, and data exchange forums. Each of these activities was started on 9/1/03. The students have attended all of the breast cancer lecture series and all of the data workshop/journal club.

Task 4. Monitor progress of past and current trainees and quality of the program. Research progress is monitored at regular research meetings by the mentors who meet weekly, by the thesis committee who meet twice per year, and by the data cub that meets biweekly. In particular the high quality of the program is assessed by the Internal Advisory Committee that meets twice a year. At its most recent meeting (8/04), the program and candidates were give excellent ratings.

Task 5. Establish a distinguished speaker series with experts in the field of breast cancer. Our goal was 2-3 speakers per year. This year we invited Dr. Nancy Colburn from NCI who lectured on cancer prevention and Dr. Richard Jove from the Moffitt Cancer Center who lectured on STAT signaling in cancer. Our annual Beckman Symposium was held on 11/04 and featured prominent speakers in the area of tumor immunology (Linda Sherman, Scripps Inst. and Pamela Ohashi, Ontario Cancer Institute). All of the fellows attended these seminars.

Key research accomplishments:
1. Graduate one trainee (Dr. Carmel Chan) from the program. He has accepted a post doc position at Sanford University working on breast cancer imaging.

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2. Conducted a formal course entitled "Breast Cancer Biology and Pathology". We also sponsored a mini-course on breast cancer clinical trials.

3. Conducted biweekly meetings with the students and mentors in a joint data/journal club in which the students present their research data at one meeting and a recent journal article on breast cancer at another.

4. Supported high quality research on breast cancer by funding seven fellows. See individual reports.

5. Sponsored breast cancer speakers, Dr. Nancy Colburn from NCI who lectured on cancer prevention and Dr. Richard Jove from the Moffitt Cancer Center who lectured on STAT signaling in cancer.

6. Supported travel for two fellows to present their work at national meetings (AACR).

Reportable outcomes:

The following are excerpts from trainee progress reports:

Examining the role of Msh2 and Mre11 in Telomere Rescue
Mentor: Dr. Adam Bailis
Fellow: Damon H. Meyer

All organisms must control recombination between non-homologous sequences to maintain genome stability. Controlling recombination at telomeres is especially important given the fact that telomere integrity, and length, are important in the regulation of cell growth. Human and yeast cells that do not express Est2, which codes for the protein subunit of telomerase needed to complete telomere replication, will ultimately arrest their growth and die as a result of telomeric loss. In humans, a small number of somatic cells faced with this crisis will stabilize their genomes by activating telomerase. These cells then have the ability to become cancerous depending on whether additional genetic changes have occurred.

Interestingly, about 15% of human cancers have an inactive telomerase gene, suggesting the presence of telomerase-independent mechanisms for regaining telomeric sequences. Recently it has been shown that both humans and *S. cerevisiae* can recover telomeric sequences by homologous recombination using a process referred to as alternative lengthening of telomeres (ALT). In *S. cerevisiae*, there are two ALT pathways controlled by Rad51 and Mre11/Rad50/Xrs2 (MRX). The ALT pathway used in humans generates telomeres that resemble the MRX-dependent survivors found in yeast. This is consistent with the observation that the human homologue of Xrs2, NBS1, is associated with telomeres only in telomerase-negative cancer cell lines. Recent evidence suggests that the mismatch repair pathway, which is thought to block recombination between mismatched telomeric sequences, limits ALT, perhaps by opposing MRX-dependent ALT.

I propose to examine the role of Msh2, the central mismatch repair protein, in restricting ALT in *S. cerevisiae*. Specifically, the role of Msh2 will be determined by examining the effect of specific *msh2* mutations on the number and types of recombination-dependent survivors, as

well as the number of generations until senescence in telomerase-deficient strains. Particular attention will be paid to whether Rad51 or MRX pathway recombinants predominate in these strains, suggesting that mismatch repair selectively restricts one pathway or the other. Understanding how mismatch repair restricts ALT will require a better understanding of how it is generated. Therefore, the role of Mre11 in ALT will be studied by determining the effect of mutations in specific functional domains of Mre11 in telomerase-independent telomere rescue. This may help determine the relationship between MRX-dependent ALT and specific homologous recombination mechanisms.

The study of the genetic and molecular control of telomere recombination and how it influences cell growth will be important in better understanding the events that lead to cancer and may ultimately help to generate attractive targets for drugs in telomerase-null breast cancers.

Publications: None.

Mechanism of Herceptin Resistance
Mentors: Dr. Susan Kane/John Shively
Fellow: Carmel Chan

Her2 is overexpressed in about 25-30% of human breast cancers. Herceptin® is a recombinant humanized Her2 antibody used to treat breast cancer patients with Her2 overexpression. Over a 5-month selection process, we isolated clones of BT474 (BT) human breast carcinoma cells (BT/Her^R) that were resistant to Herceptin *in vitro*. The resistance phenotype remained stable after BT/Her^R subclones were cultured in the absence of Herceptin for 4-5 months. The level of cell-surface and total EGFR was also similar between BT/Her^R subclones and BT cells whereas the level of total Her3 was upregulated in some of the BT/Her^R subclones. In the presence of Herceptin, phosphorylated and total Akt and ERK1/2 levels were partially sustained in BT/Her^R subclones, but not in BT cells. Moreover, BT/Her^R subclones showed sustained sensitivity to the PI-3K inhibitor LY294002 and Rapamycin in the presence of Herceptin, whereas BT cells were rendered insensitive to LY294002 and Rapamycin once they were growth inhibited by Herceptin. Taken together, these last two observations suggest that BT/Her^R subclones acquired Herceptin-resistant PI-3K signaling and that this pathway was at least partially responsible for the growth of BT/Her^R subclones in the presence of Herceptin. Herceptin also sensitized BT/Her^R subclones to the EGFR kinase inhibitor AG1478, consistent with a partial shift towards EGFR signaling in the presence of Herceptin. Our BT/Her^R subclones should be useful for understanding the mechanisms of Herceptin resistance in breast cancer patients.

Publications:

Chan, C.T., Metz, M.Z. and Kane, S.E. 2003. Characterization of Herceptin®-Resistant human breast carcinoma cells. Breast Cancer Research and Treatment, in press.

In vivo Imaging of *mdr*1a Gene Expression.
Mentors: Drs. Susan E. Kane and Timothy Synold
Fellow: Donna A. Brown

Despite advances in the understanding of transcriptional regulation of *MDR*1 expression *in vitro*, in the context of the whole body, the regulatory mechanisms of *MDR*1 expression are poorly understood. Our lack of understanding is due to our inability to measure changes in gene expression under real physiological conditions and in real time, and until now good models have not been available. Using small animal imaging tools we have designed a mouse model that will allow us to examine *mdr*1a (a homologue of human *MDR*1) expression *in vivo*, in real time, and in response to developmental, physiological, and environmental signals, with a view to resolving unanswered questions pertaining to the over-expression of *MDR*1 and clinical drug resistance.

Through homologous recombination we have inserted the *Renilla* luciferase (LUC) gene into the *mdr*1a genomic locus and thus under the control of the *mdr*1a promoter. Cre-loxP technology was incorporated into the model such that in-frame expression of the LUC reporter is conditional on Cre-mediated recombination and *mdr*1a expression. By controlling Cre expression we can examine tissue-specific expression of *mdr*1a. To our knowledge our model is unique and therefore, if successful, will be the first system of its kind to examine locus controlled, regulated reporter gene expression in specific tissues *in vivo*.

We have created mouse embryonic stem (ES) cells and demonstrated homologous recombination of our targeting vector into the mouse *mdr*1a genomic locus; the ES cells were used to generate knock-in mice that have been crossed with ubiquitous Cre-donator mice. Mice positive for Cre-recombination have been analyzed for LUC expression using Xenogen's IVIS® imaging system and from 14 mice screened, one mouse was positive for LUC signal in the abdominal region. Treating mice with drugs known to induce *mdr*1a had no effect on LUC expression. To ensure that lack of signal was not due to a sensitivity issue, LUC expression was examined *ex vivo* in tissues where *mdr*1a expression is normally observed. Although LUC expression was detected in the stomach of one mouse, it appears unlikely that *in vivo* expression of LUC was undetectable due to sensitivity limitations. To confirm this hypothesis RT-PCR will be used to analyze *mdr*1a and LUC expression in dissected organs. The positive LUC signal in one of our mice may be associated with an inflammatory response; this is being investigated in light of potential implications for induced *mdr*1a expression under physiological stress conditions.

To optimize LUC expression, we have redesigned the targeting vectors; vectors now contain either the humanized *Renilla* (optimal codon usage for mammalian systems) or firefly luciferase reporter genes. The Cre-lox system has been utilized to remove the neo selection marker (presence of the neo gene can affect endogenous gene expression) prior to generating transgenic mice. Synthetic polyA signals have also been incorporated into the new vectors. It is hoped that these targeting vectors will result in detectable basal levels of luciferase expression in knock-in mice.

Abstracts:
Donna A. Brown, Susan E. Kane and Timothy Synold. (2003) *In vivo* Imaging of *mdr*1a Gene Expression. Abstract for the Pathobiology of Cancer Workshop, Keystone, Colorado.

Brown, D.A., Kane, S.E., Synold, T.W. (2004) Genetic controlled reporter gene expression. The Third Annual Meeting of The Society for Molecular Imaging, St Louis, Missouri.

Meetings:
Pathobiology Cancer Workshop, Keystone, Colorado. July 13-20 2003.

The Second Annual Meeting of Molecular Imaging, San Francisco, California. August 15 –18 2003.

Alternative messenger RNA splicing and breast cancer
Mentor: Dr. RJ Lin
Fellow: Chunxia Li

Alternative splicing is a key post-transcriptional mechanism to contribute to the human genome complexity. Growing evidence indicates that alternative or aberrant pre-mRNA splicing takes place during the development, progression, and metastasis of breast cancer. For example, splice variants of several genes including estrogen and prolactin receptors, BRCA-1, CD44, Her2/neu, AIB-1 and VEGF are detected in breast cancer cell lines or tumor tissues. To profile the splicing pattern changes in breast cancer cells, we use microarray techniques to simultaneously assay splicing variations of a number of known human genes implicated in cancer progression and apoptosis. The customized microarrays contain oligonucleotides designed to detect alternatively spliced mRNA variants.

We compared breast cancer cell line MDA-MB-231 (Estrogen receptor negative cell line) and MCF7 (ER positive cell line) cells with cultured human mammary epithelial cells (HMEC) and found some common splicing changes including CD44, FAS, LARD, RBM9, HnRNPA/B, APLP2, and MYL6 by the microarray, which were further verified by RT-PCR. We also compared splicing in the two breast cancer cells cultured in either two-dimensional flat dishes (2-D) or in three-dimensional Matrigel (3-D) conditions. Interestingly, only a subset of the splicing differences that distinguish MCF7 cells from MDA-MB-231 cells under 2-D culture condition is retained under 3-D conditions, suggesting that alternative splicing events are regulated by culture condition. On the other hand, one gene MYL6 (myosin light chain) is spliced differently in MCF7 cells when cultured in different systems. Furthermore, using RT-PCR, we found alternative splicing in several genes including MYL6 in MCF7 cells cultured in 3-D is most similar to that from MCF7-derived tumors in nude mice. Now we are focused on studying the regulations involved in these splicing changes between two different breast cancer cells.

Meetings/Abstracts:
1. DOD breast cancer meeting (Sep 2002, Orlando FL)
Chunxia Li, Valerie Welch, Manuel Ares, Jr., and Ren-Jang Lin
Investigation of Alterations in Pre-mRNA Splicing in Breast Cancer Using Oligonucleotide Microarrays

2. Cold Spring Harbor eukaryotic mRNA processing meeting (Aug 2003, CSHL NY)
Chunxia Li, Mitsuo Kato, Valerie Welch, Manuel Ares, Jr., and Ren-Jang Lin   Investigation of Alterations in Pre-mRNA Splicing in Breast Cancer

3. RNA Society annual meeting (Jun 2004, Madison WS)
Chunxia Li, Mitsuo Kato, Manny Ares, Jr., Ren-Jang Lin
Identifying splicing changes between breast cancer cells using splicing-sensitive oligonucleotide microarrays

Inhibition of nuclear receptors by the anticancer drug ET-743
Mentor: Dr B.M. Forman
Fellow: Dr Corinne Solier

Current treatments of breast cancer involve antiestrogen therapies, including the commonly used tamoxifen. Unfortunately, 90% of women treated with this drug acquire resistance to tamoxifen within 1 year. Moreover, there are side effects associated with tamoxifen, including increased risk of endometrial cancer, which indicates the requirement to improve breast cancer treatments. Ecteinascidin-743 (ET-743), a novel natural marine molecule currently undergoing Phase II / III clinical trials, is an extremely potent anticancer drug acting on a broad panel of tumors, including breast cancer sarcoma. It is of note that a structural analog of ET-743, phthalascidin (PT-650), which displays similar antiproliferative properties as ET-743, has recently been synthesized. The mechanisms of action of ET-743 still remain unclear.

We previously showed that ET-743 / PT-650 inhibit specifically in a broad-specific manner the ligand-induced but not the basal activity of nuclear and non-nuclear pathways. We also showed that the effects on hormone receptors are mediated, at least in part, by the displacement of coactivator molecules and the concomitant recruitment of corepressors. Fluorescence Resonance Energy transfer (FRET) assays allowed us to show that the displacement of coactivators occur within 15 to 30 minutes but Electrophoretic Mobility Shift Assays (EMSA) ruled out a potential direct effect of ET-743 / PT-650 on the association between hormone receptors and coactivators. These data suggested that ET-743 / PT-650 mediate their transcriptional inhibitory effects by targeting a step common to many transcription-activation patways. We reasoned that ET-743 / PT-650 may induce a modification of the phosphorylation pattern of the RNA polymerase II carboxy-terminal domain (CTD), which is critical for RNA polymerase II's ability to initiate transcription and elongate the message.

We next showed by western blot analysis of cell extracts from CV-1 and 293-T cells treated with ET-743 / PT-650 that these drugs increase phosphorylation of RNA polymerase II CTD on both Ser-2 and Ser-5 of the heptapeptide (YSPTSPS) repeats. This effect was inhibited by flavopiridol, a P-TEFb-selective inhibitor (obtained from the National Cancer Institute (NCI) reagent depository), suggesting that ET-743 / PT-650 could target the activity of this RNA polymerase II-specific kinase. We conducted in vitro kinase assays using a large panel of RNA polymerase II-specific kinases, which established that ET-743 upregulates specifically P-TEFb (provided by Dr D. Price, University of Iowa) kinase activity. To further delineate the in vivo effect of ET-743 / PT-650 on the kinase activity of P-TEFb, we analyzed the effects of these compounds on the association of P-TEFb (CDK9 / cyclin T1 or T2) with 7SK snRNA, a small nuclear RNA inhibiting specifically P-TEFb in association with HEXIM1 / MAQ1 coregulator. For this, we immunoprecipitated P-TEFb from extracts from 293T cells treated with the drugs and assessed the binding of 7SK snRNA to immunoprecipitated P-TEFb by real-time quantitative PCR: data showed that ET-743 / PT-650 dissociate 7SK snRNA from P-TEFb in a dose-responsive manner and within 30 minutes of treatment. This correlated with an increased kinase activity of P-TEFb, which confirms that P-TEFb is activated by ET-743 / PT-650 in vivo.

Further investigations are under way to study whether an upregulation of P-TEFb by ET-743 / PT-650 accounts for the transcriptional inhibitory effects of these drugs.

Human splicing factor Prp2 as a molecular target of breast cancer treatment.
Mentor: Dr. RJ Lin
Fellow: Dr. Mitsuo Kato

Pre-mRNA processing is very important in cell growth and cell survival, because it is a key step in gene expression and regulation. Several recent reports suggest that some splicing factors are associated with breast cancer development. Prp2 helicase is an essential factor required for the first transesterification of splicing reaction in yeast *S. cerevisiae*. A human protein hPrp2 (DHX16/DBP2/KIAA0577) with extensive homology to *S. cerevisiae* Prp2 was identified. We found that hPrp2 functions in splicing, and dominant negative mutants induce cell growth arrest and apoptosis in human embryo kidney cell line (HEK293). The expression level of hPrp2 in a number of breast cancer cell lines was much lower than that in human mammary epithelial cells (HMEC). Since deletions of the chromosome region carrying the hPRP2 gene were often found in breast cancer, hPrp2 may have a tumor suppressor function. We found that introduction of the hPRP2 gene to breast cancer cell line MCF7 induced apoptosis. The N-terminus of hPrp2 was indispensable for the induction of apoptosis. Treatment of caspase-3 inhibitor DEVD-CHO inhibited apoptosis but not cell growth arrest. Thus, human Prp2 may regulate cell growth and cell death in different pathways, and cell death is partially mediated by caspase-3.

By using RT-PCR and microarray, we detected changes in splicing and in some cases changes in the expression of intronless genes, suggesting that hPrp2 may regulate gene expression at multiple steps. Because estrogen response is very important for breast cancer development, effects of hPrp2 expression on estrogen response were studied using luciferase reporter that is regulated by an estrogen responsive element. Interestingly, hPrp2 repressed estrogen response and the repression was altered by mutations of the helicase domain. In addition, hPrp2 repressed the expression from aromatase promoter. These results suggest that hPrp2 have similar effect like Tamoxifen (ER inhibitor) and Letrozol (aromatase inhibitor), both of which are currently used for breast cancer treatment.

Further analysis of hPrp2 on transcription revealed that wild-type hPrp2 inhibits luciferase reporter activity driven from some promoters, however helicase domain mutants of hPrp2 enhance the reporter activity. When the expression of reporters was assayed by RT-PCR, the mRNA level decreased with wild-type hPrp2 but no obvious change of mRNA was observed with helicase domain mutants. Decrease in steady state mRNA with wild-type hPrp2 is

consistent with the result of the reporter activity. The control of the expression of wild-type hPrp2 could be important for breast cancer. In addition, it is intriguing that helicase domain mutants induced reporter activity without increase of mRNA. This result suggests that helicase domain mutants may enhance mRNA export or translation.

We are currently investigating the mechanism through which hPrp2 induces apoptosis and exploring the feasibility to use hPrp2 as a molecular target for breast cancer treatment.

Meetings/abstracts:

Kato M, Gopalakrishna S, Chavez V, Silverman E, Li C, Gaur R, & Lin R-J: Involvement of human Prp2 in Pre-mRNA Splicing and Spliceosome Cycle. Ninth Annual Meeting of the RNA Society, Madison, WI, USA, June 1-6, 2004.

Li C, Kato M, Ares M Jr & Lin R-J: Detection using oligonucleotide microarray of splicing differences and the involvement of SR proteins in breast cancer cells. Ninth Annual Meeting of the RNA Society, Madison, WI, USA, June 1-6, 2004.

Study of the Pharmacokinetic Properties and Therapeutic Potential of scFv-Fc Fragments in a Metastatic Breast Cancer Mouse Model
Mentor: Dr. Anna Wu
Fellow: Vania E. Kenanova

Genetically engineered antibody fragments possess the potential for implementation in cancer therapy and imaging as tumor specific delivery agents. In this study, we selected the scFv-Fc [(scFv-C_H2-$C_H3_{\gamma1}$)$_2$, 105 kDa] format since it has been shown to exhibit tumor uptake and serum clearance kinetics similar to the intact IgG1. The serum persistence of intact IgG and fragments with complete Fc region is controlled by interactions with the protective FcRn receptor. We developed five anti-carcinoembryonic antigen (CEA) scFv-Fc variants (I253A, H310A, H435Q, H435R and H310A/H435Q) with mutations in the Fc:FcRn binding site in order to modulate their clearance rates. We recently reported biodistribution studies in Balb/c mice using ^{125}I or ^{131}I-labeled fragments which revealed blood clearance rates from the longest to the shortest as follows: wild type > H435R > H435Q > I253A > H310A > H310A/H435Q. The biodistribution studies in Balb/c mice showed terminal half-lives of the mutant scFv-Fcs ranging from 83.4 to 7.96 hours. Additionally, dual biodistribution studies with ^{125}I/^{111}In-DOTA-conjugated I253A, H310A and H310A/H435Q in LS174T xenograted athymic nude mice measured radiometal maximum tumor uptake of 44.6, 33.7 and 28.0% ID/g for the I253A, H310A and H310A/H435Q fragments respectively. The same radioiodine conjugated fragments achived lower maximum tumor activity. The liver and kidney uptake ranged from 10 - 20% ID/g and 4 – 6% ID/g respectively, demonstrating primary clearance through the liver. Furthermore, [124]I-labeled scFv-Fc fragments were also evaluated in LS174T xenografted athymic mice by microPET, revealing localization to the CEA-positive xenografts and low activity in normal organs. In addition, microPET imaging by ^{64}Cu-DOTA-conjugated I253A, H310A and H310A/H435Q proteins allowed for quantitation of radiometal-labeled antibody uptake. The ^{64}Cu microPET quantitation of tumor and organ uptake correlated with the ^{111}In biodistribution studies in tumor bearing mice. CEA is expressed by more than 50% of breast cancers, while the normal CEA expression is limited to the colon lumen. Based on these data we decided to evaluate the therapeutic potential of the mutant constructs I253A and H310A, intermediate in blood activity, for the therapy of metastatic breast cancer. For the establishment and validation of a metastatic breast cancer model, MCF-7/Her2/Renilla Luciferase and MCF-7/Her2/rLuc/CEA (CEA overexpressing MCF-7/Her2 cells) were generated for the development of CEA-positive breast cancer metastases. As a control, a CEA-negative

breast cancer metastatic cell line MDA-MB-231/rLuc was also generated. The ability of the transfected cells lines to produce tumors in nude mice was evaluated by generation of subcutaneous xenografts. Mice with visible tumors were then tail-vein-injected with coelecterazine (Renilla luciferase substrate) and the output of light from the tumors was optically imaged using a charge-coupled device (CCD) camera. Following these preliminary experiment, each cell line will be introduced into athymic nude mice by three alternative routes – intravenous (lung seeding), orthotopic (mammary fat pad) and osteosclerotic (left ventricle heart injection). The dispersal of tumor cells and the formation of metastases will be confirmed by measuring the output of light produced by the Renilla luciferase via a CCD camera system. Histological and histochemical tissue examinations will be performed to independently validate the metastatic animal models. For the therapy studies in the established metastatic breast cancer animals, the scFv-Fc variants selected for therapy will be labeled either with Y-90 or Lu-177. Mice will undergo treatment and their response will be evaluated through the use of a multimodality approach incorporating optical, microPET and microSPECT systems. MicroPET imaging will involve ^{18}F-FDG, ^{18}F-FLT and ^{124}I/^{64}Cu labeled scFv-Fcs, while microSPECT imaging will utilize ^{99m}Tc-Annexin V and Lu-177. The pharmacokinetic properties of scFv-Fcs will further be studied in human FcRn transgenic mice. Our primary goal is to demonstrate that CEA is a valid metastatic breast cancer target and that larger recombinant fragments resembling intact antibodies (both structurally and functionally) have enhanced tumor uptake when compared to smaller fragments, suggesting utility in metastatic breast cancer treatment.

Meetings:
Therapeutic Antibodies, Inforcast, June 22 and June 24 in San Diego, CA.

Additional reportable outcomes (past trainees):
Publications:

Kirshner, J., Chen, C-J., Liu, P., Huang, J., and Shively, J.E. CEACAM1-4S, a cell-cell adhesion molecule, mediates apoptosis and reverts mammary carcinoma cells to a normal phenotype in a 3D culture. **Proc. Natl. Acad. Sci. USA. 100:** 521-526, 2003.

Kirshner, J., Schumann, D., and Shively, J.E. CEACAM1, a cell-cell adhesion molecule, directly associates with annexin II in a 3D model of mammary morphogenesis. **J. Biol. Chem., 278:** 50338-50345, 2003.

Conclusion:
We have accomplished our goals for the training program. In the past year we supported 7 fellows and the program ended on 09/1/04. The research accomplishment of the fellows was very good with a number of manuscripts already published and several more in preparation.

References:
    N/A

Appendix I

BREAST CANCER JOURNAL/DATA CLUB 2003 - 2004
Library Conference Room
4:00 – 5:00 P.M.

<u>2003</u>

October 7   Data   Carmel Chan
"Role of Phophatidylinositol-3 kinase pathway in trastuzumab (Herceptin)-Resistant Human Breast Cancer Cells"

October 21   Journal   Chunxia Li
"Regulation of the activity of p38 mitogen-activated protein kinase by Akt in cancer and adenoviral protein E1A-mediated sensitization to apoptosis"

November 4   Data   Mitsuo Kato
"Function of RNA helicase hPrp2 in splicing and Breast Cancer"

November 18   Journal   Corinne Solier
"The BRCT domain is a phospho-protein binding domain"

December 2   Data   Damon Meyer
"BRCA2-dependent and independent formation of RAD51 nuclear foci"

December 16   Journal   Vania Kenanova
"Study of the Pharmacokinetic Properties of scFv-Fc Fragments in a Metastatic Breast Cancer Mouse Model"

<u>2004</u>

January 6   Data   Donna Brown
"*In Vivo* Imaging Of *Mdr*1a Gene Expression In Breast Cancer"

January 20   Journal   Jennifer Murray
"Additive antitumour effect of the epidermal growth factor receptor tyrosine kinase inhibitor gefitinib (Iressa, ZD1839) and the antioestrogen fulvestrant (Faslodex, ICI 182,780) in breast cancer cells"

February 3   Data   Chunxia Li
"Investigating the splicing variations in breast cancer"

February 17   Journal   Mitsuo Kato
"Aberrant splicing induced by missense mutations in BRCA1: clues from a humanized mouse model"

March 2   Data   Corinne Solier
"Inhibition of nuclear receptors by the anti-cancer drug Ecteinascidin-743 (ET-743)"

March 16   Journal   Carmel Chan
"The heat shock protein 90 inhibitor geldanamycin and the ErbB inhibitor ZD1839 promote rapid PP1 phosphatase-dependent inactivation of Akt in ErbB2 overexpressing breast cancer cells"

March 30   Data   Damon Meyer
"Examining the role of *MSH2* and *MRE11* in telomere rescue"

April 13   Journal   Vania Kenanova
"Conditional activation of Neu in the mammary epithelium of transgenic mice results in reversible pulmonary metastasis"

April 27   Data   Jennifer Murray
Cancelled

May 11   Journal   Donna Brown
"Cross-resistance studies of isogenic drug-resistant breast tumor cell lines support recent clinical evidence suggesting that sensitivity to paclitaxel may be strongly compromised by prior doxorubicin exposure"

May 25   Data   Limin Liu
"The taxol-like activity of the RASSF1A tumor suppressor protein"

Appendix II
Breast Cancer Biology Course 2003

9/17	Oncogenes and breast cancer	Dr. J.K. Yee
9/24	Apoptosis, Survival and Response to Therapy	Dr. W. Chow
10/1	Hormone and breast cancer	Dr. S. Chen
10/8	Growth factors and signal transduction	Dr. Natarajan
10/15	Tumor suppressor genes	Dr. G. Pfeifer
10/22	Basic anatomy, physiology and pathology of breast cancer	Dr. Wilczynski
10/29	Clinical pharmacology of breast cancer	Dr. Synold
11/5	Breast cancer treatment	Dr. L. Leong
11/12	Tumor Angiogenesis	Dr. Wei Wen
11/19	Cell-cell and cell-matrix interaction, metastasis	Dr. J. Shively

Other information

1. There are ten lectures. We meet once a week (5:30 pm to 7:30 pm, Wednesday) at the Conference room in the second floor of the Kaplan Clinical Research Building.
2. The students will be provided with the slide printout and an outline of the lecture.
3. Each instructor will provide a question. The students are required to choose a question and write a 5-page term paper which is due on December 3.
4. Contact Shiuan Chen (x63454) if you have any questions.